Gram staining

Slide
↓
1 drop normal saline
↓
a loop of bact. colony
↓
air day
↓
Heat fix
↓
methylene blue/ Crystal violet/ gentian violet for 1 min.
↓
Iodine 1 min
↓
wash with water
↓
Acetone/ alcohol 2-3 sec.
↓
wash
↓
Counterstain carbol fuschin/ neutral red/ safranin 1-2 min
↓
wash → dry → oil immersion → 100X

Principle :
- Primary stain (basic) + Iodine → insoluble complex in cytoplasm
- decolourisers dissociate this compound and remove from cell. slower in G+ve.

Reason :- (1) G+ve have thicker & dense peptidoglycan cell wall which is less permeable
(2) G+ve have acidic cytoplasm to bind more firmly with basic dye.

G+ve look like G-ve when → overdecolorisation
→ rupture of cell wall during heat fixation

G-ve look like G+ve when → underdecolorisation

G+ve cocci (violet, rounded)	G+ve bacilli (violet, rod)
→ Staphylococci	→ M. tuberculosis
Streptococci	→ M. leprae
Micrococci	→ Clostridium
Pneumococci	→ Corynebacterium
enterococci	→ Bacillus Anthracis
G-ve cocci (Pink, round)	**G-ve bacilli (Pink, rod)**
→ Neisseria meningitidis	→ E. coli
→ Neisseria gonorrhoea	Proteus
→ moraxella	Pseudomonas
	Salmonella
	Shigella

Crystal violet sol^n = Crystal violet (0.5gm) + dist. water (100
Gram's iodine = Iodine (1gm) + KI (2gm) + dist. water (100ml)
safranine = Safranine (0.5gm) + dist. water (100 ml)

Teacher's Signature

Notes

Zn Staining (Acid fast)

Heat fixed slide
↓
Carbol fuschin
↓
Heat till steam arises
↓
wait 5 min.
↓
wash
↓
20% H_2SO_4 1 min or
↓
wash
↓
repeat this till pink/red colour stop coming
↓
2% methylene blue 1 min. or malachite green
↓
wash
↓
dry → oil immersion → 100x

AF +ve →
- M. tuberculosis
- M. leprae (need 5% H_2SO_4)
- Nocardia (need 1% H_2SO_4)
- Spore
- Head of sperm

Brick red bacilli with blue background. (blue coloured Pus cells may be seen)

Principle :- mycolic acid (α Hydroxy, long chain fatty acid) present in cell wall so heat is applied for penetration of carbol fuschin and mycolic acid is also responsible for resist to decolorisor.

Ziehl-Neelsen's carbol fuschin :-
(Basic fuschin (1 gm) + Phenol (5 gm) + Alcohol (95%) → 10 ml, distill water. → 100 ml)

Grading of smear (RNTCP) :-

No. of AFB seen in oil immersion fields	Report
0/300 field	No AFB
1-2/300 field	doutful, repeat
1-9/100 field	1+
1-9/10 field	2+
1-9/1 field	3+
10-more/1 field	4+

Notes

Negative staining

Principle :- Indian Ink / negrosin (acidic)

- chromophore (-ve ion) → responsible for staining → stain only background bcz cell wall of bacteria is also -ve charge so they repel chromophore
- H^+ ion

Dry method

Slide
↓
a drop india Ink / negrosine
↓
1 loop colony
↓
mix
↓
spread with another slide (like PBF)
↓
dry
↓
oil immersion
↓
100X

eg. Streptococcus pneumoniae
Crypto coccus neoformans
H. influenzae
Klebsiella
Syphillis

→ they seen bright unstained against dark background.

Spore staining

Page No.:
Date: / /

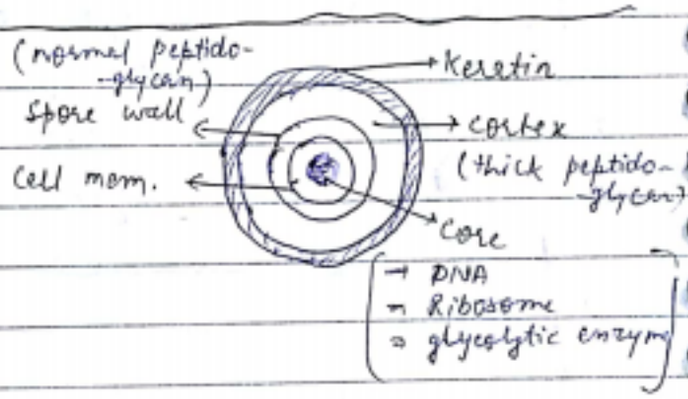
Notes

Heat fixed slide
↓
malachite green (5%)
↓
5-6 min. heat till steam arises
↓
wash
↓
(0.5%) basic fuschin for 30 sec.
↓
wash dry
↓
oil immersion
↓
100X

⇒ Spore stain green and bacillus stain red

- spore →
  - Central ← oval / spherical → Non bulging
  - Terminal
    - oval → Cl. tertium
    - spherical → Cl. tetani
    - → Bulging
  - Sub terminal

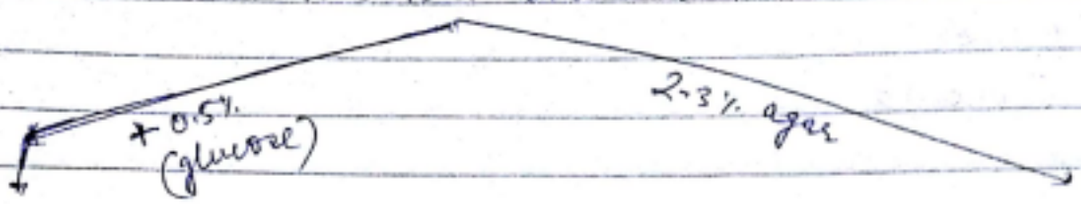
eg: Central → Bacillus anthracis
Sub terminal → Cl. perfringes



⇒ Spore represent a dormant resting stage in growth cycle of organism.

Notes

⇒ Nutrient Broth (Pepton water + 1% meat extract)



Glucose Broth

Nutrient agar

⇒ Trichophyton mentagrophytes :- Clusters of microconidia
→ macroconidia ∮ cigar shaped with terminal rat tail filaments

⇒ Aspergillus fumigatus :- ~~Con~~ vesicle is conical shaped
→ ~~phiati~~ Phialides arranged in single row.
→ Conidia arises from upper 1/3rd vesicle.

Aspergillus flavus → whole ~~conidi~~ vesicle

Aspergillus niger

Hyaline conidia

Black conidia

γ	β	γ
⇒ Strep pneumoniae Strep. viridans	Strep. pyogens	Staph. epidermidis Staph. saprophyticus

⇒ Penicillin → 1 stage branch
2 " →
3 " →